

We claim:

1. A cryogenically protected viral delivery system for infecting host cells comprising a cryogenic vessel and a plurality of virally infected cells in admixture with a cryo-protective agent contained in the cryogenic vessel,
5 wherein the concentration of virally infected cells is from 10^6 cells/ml to 10^9 cells/ml;
wherein the admixture of the virally infected cells and the cryo-protective agent is at a temperature of less than or equal to $-20\text{ }^{\circ}\text{C}$;
10 and
wherein the viability of cells contained in the cryogenic vessel is at least 50%.
2. The cryogenically protected viral delivery system according to claim 1,
15 wherein the viability of the cells is at least 70%.
3. The cryogenically protected viral delivery system according to claim 1, wherein the viability of the cells is at least 90%.
- 20 4. The cryogenically protected viral delivery system according to claim 1, wherein the admixture is substantially free of extracellular viral particles.
5. The cryogenically protected viral delivery system according to claim 1, wherein the admixture is substantially free of spent incubation media.
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6. The cryogenically protected viral delivery system according to claim 1, wherein the average cell diameter of cells contained in the cryogenic vessel is at least $0.5\text{ }\mu\text{m}$ greater than the average cell diameter of uninfected cells of the same type.
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7. The cryogenically protected viral delivery system according to claim 1, wherein the admixture of virally infected cells and cryo-protective agent is at a temperature of less than or equal to $-70\text{ }^{\circ}\text{C}$.

8. The cryogenically protected viral delivery system according to claim 1, wherein the admixture of virally infected cells and cryo-protective agent is at a temperature of less than or equal to -130°C .
- 5 9. The cryogenically protected viral delivery system according to claim 1, wherein the cryo-protective agent is selected from the group consisting of DMSO, serum albumin, serum, glycerol, and mixtures thereof.
- 10 10. The cryogenically protected viral delivery system according to claim 1, wherein the cryogenic vessel contains from 10^5 to 10^{12} virally infected cells.
11. The cryogenically protected viral delivery system according to claim 1, wherein the volume of the cryogenic vessel is less than or equal to 250 ml.
- 15 12. The cryogenically protected viral delivery system according to claim 1, wherein the volume of the cryogenic vessel is less than or equal to 30 ml.
13. The cryogenically protected viral delivery system according to claim 1, wherein the volume of the cryogenic vessel is less than or equal to 6 ml.
- 20 14. The cryogenically protected viral delivery system according to claim 1, wherein the cryogenic vessel is a polypropylene vial having a volume of less than or equal to 6 ml.
- 25 15. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells in the vessel represent at least 20% of the total number of cells in the vessel.
- 30 16. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells in the vessel represent at least 40% of the total number of cells in the vessel.

17. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells in the vessel represent at least 60% of the total number of cells in the vessel.
- 5 18. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are eukaryotic cells.
19. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are insect cells.
- 10 20. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are infected with recombinant virus.
- 15 21. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are infected with a virus selected from the group consisting of baculovirus, adenovirus, adeno-associated virus, influenza virus, canarypox virus, infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, parainfluenza 3 virus, bovine respiratory syncytial virus, feline calicivirus, chlamydia virus, canine coronavirus, panleukopenia virus, feline leukemia virus, hepatitis A, hepatitis B, hepatitis C, human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), cytomegalovirus, human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II), encephalitis virus, measles virus, mumps virus, rubella virus, polio virus, rabies virus, respiratory syncytial virus, rotavirus, smallpox virus, typhoid vaccine virus, varicella virus, yellow fever vaccine virus, and combinations thereof.
- 20 22. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are infected with baculovirus.
- 25 23. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are infected with adenovirus.
- 30 24. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are infected with adeno-associated virus.

25. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are infected with influenza virus.
- 5 26. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are Sf9 cells infected with recombinant baculovirus.
- 10 27. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are Sf9 cells infected with recombinant baculovirus carrying a heterologous polynucleotide operatively linked to a baculovirus polyhedrin promoter.
- 15 28. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are mammalian cells infected with adenovirus.
- 20 29. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are HEK-293 cells infected with adenovirus.
- 30 30. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are HEK-293 cells infected with recombinant adenovirus.
- 25 31. The cryogenically protected viral delivery system according to claim 1, wherein the virally infected cells are mammalian cells infected with influenza virus.
- 30 32. An intermixture of virally infected cells and uninfected host cells obtained by:
providing a plurality of frozen virally infected cells obtained from the admixture contained in the cryogenically protected viral delivery system of claim 1;
providing a plurality of uninfected host cells, wherein the concentration of the uninfected host cells is from 10^5 cells/ml to 10^7 cells/ml;

optionally thawing, washing and/or lysing the virally infected cells; and inoculating at least a portion of the uninfected host cells with at least a portion of the virally infected cells.

- 5 33. The intermixture according to claim 32, wherein the viability of cells obtained from the admixture contained in the cryogenically protected viral delivery system of claim 1 is at least 70%.
- 10 34. The intermixture according to claim 32, wherein the viability of cells obtained from the admixture contained in the cryogenically protected viral delivery system of claim 1 is at least 90%.
- 15 35. The intermixture according to claim 32, wherein the temperature of the virally infected cells obtained from the admixture contained in the cryogenically protected viral delivery system of claim 1 is less than or equal to -70 °C.
- 20 36. The intermixture according to claim 32, wherein the temperature of the virally infected cells obtained from the admixture contained in the cryogenically protected viral delivery system of claim 1 is less than or equal to -130 °C.
- 25 37. A method for preparing a cryogenically protected viral delivery system comprising:
admixing a plurality of virally infected cells with a cryo-protective agent to obtain an admixture having a concentration of virally infected cells of from 10^6 cells/ml to 10^9 cells/ml; and
freezing at least a portion of the admixture for a time and under conditions sufficient so that the temperature of the frozen admixture is less than or equal to -20 °C and so that the viability of the cells in the frozen admixture is at least 50%.
- 30 38. The method according to claim 37, wherein at least a portion of the admixture is frozen for a time and under conditions sufficient so that the viability of the cells in the frozen admixture is at least 70%.

39. The method according to claim 37, wherein at least a portion of the admixture is frozen for a time and under conditions sufficient so that the viability of the cells in the frozen admixture is at least 90%.
- 5 40. The method according to claim 37, wherein the admixture is substantially free of extracellular viral particles.
41. The method according to claim 37, wherein the admixture is substantially free of spent incubation media.
- 10 42. The method according to claim 37, wherein at least a portion of the admixture is frozen for a time and under conditions sufficient so that the temperature of the frozen admixture is less than or equal to -70°C .
- 15 43. The method according to claim 37, wherein at least a portion of the admixture is frozen for a time and under conditions sufficient so that the temperature of the frozen admixture is less than or equal to -130°C .
- 20 44. The method according to claim 37, wherein at least a portion of the admixture is frozen by reducing the temperature at a rate of from $1^{\circ}\text{C}/\text{minute}$ to $30^{\circ}\text{C}/\text{minute}$.
- 25 45. The method according to claim 37, wherein the plurality of virally infected cells and the cryo-protective agent are admixed in amounts to obtain an admixture having a concentration of virally infected cells of from 5×10^6 cells/ml to 5×10^8 cells/ml.
- 30 46. The method according to claim 37, wherein the plurality of virally infected cells are admixed with a cryo-protective agent selected from the group consisting of DMSO, serum albumin, serum, glycerol and mixtures thereof.
47. The method according to claim 37, further comprising preparing the plurality of virally infected cells by:
inoculating a plurality of uninfected host cells with a plurality of viruses;

incubating the inoculated cells in a composition comprising incubation media for a time and under conditions sufficient to obtain a plurality of virally infected cells; and

5 separating the plurality of virally infected cells from substantially all spent incubation media and extracellular viral particles and collecting the plurality of virally infected cells in a vessel.

10 48. The method according to claim 47, wherein the plurality of virally infected cells are collected in a vessel having a size of at least 100 ml.

49. The method according to claim 37, further comprising aliquoting at least a portion of the admixture into cryogenic vessels prior to freezing the aliquoted admixture.

15 50. The method according to claim 49, wherein at least a portion of the admixture is aliquoted into cryogenic vessels having a volume of less than or equal to 6 ml.

20 51. The method according to claim 50, wherein the cryogenic vessels are polypropylene vials.

25 52. The method according to claim 49, wherein at least a portion of the admixture is aliquoted into the cryogenic vessels in an amount from 10^6 to 10^9 virally infected cells.

53. The method according to claim 49, wherein the volume of the admixture aliquoted into the cryogenic vessels is from 0.5 ml to 20 ml.

30 54. The method according to claim 37, wherein the virally infected cells are eukaryotic cells.

55. The method according to claim 37, wherein the virally infected cells are insect cells.

56. A method of virally infecting cells comprising:
providing a plurality of virally infected cells in admixture with a cryo-protective agent contained in a cryogenic vessel;
wherein the concentration of virally infected cells is from 10^6 cells/ml to 10^9 cells/ml;
wherein the admixture of the virally infected cells and the cryo-protective agent is at a temperature of less than or equal to -20°C ; and
wherein the viability of cells contained in the cryogenic vessel is at least 50%;
- providing a plurality of uninfected host cells, wherein the concentration of uninfected host cells is from 10^5 cells/ml to 10^7 cells/ml;
optionally thawing, washing and/or lysing the virally infected cells;
inoculating at least a portion of the uninfected host cells with at least a portion of the virally infected cells; and
incubating the inoculated cells in the presence of incubation media for a time and under conditions sufficient to provide a composition in which at least 20% of the cells in the composition are virally infected.
57. The method according to claim 56, wherein the number of virally infected cells added to the uninfected host cells represent no more than 10% of the total number of cells.
58. The method according to claim 56, wherein the number of virally infected cells added to the uninfected host cells represent no more than 1% of the total number of cells.
59. The method according to claim 56, wherein the viability of cells contained in the cryogenic vessel is at least 70%.
60. The method according to claim 56, wherein the viability of cells contained in the cryogenic vessel is at least 90%.

61. The method according to claim 56, wherein the admixture of virally infected cells and cryo-protective agent contained in the cryogenic vessel is at a temperature of less than or equal to -70°C .
- 5 62. The method according to claim 56, wherein the inoculated cells are incubated for a time and under conditions sufficient so that at least 50% of the cells in the composition are virally infected.
- 10 63. The method according to claim 56, wherein the inoculated cells are incubated for a time and under conditions sufficient so that the concentration of viruses in the composition is from 10^7 viruses/ml to 10^{11} viruses/ml.
- 15 64. The method according to claim 56, wherein the inoculated cells are incubated for a time and under conditions sufficient so that the concentration of polypeptide in the composition is from 0.0001 mg/ml to 10 mg/ml.
65. The method according to claim 56, wherein the virally infected cells are eukaryotic cells.
- 20 66. The method according to claim 56, wherein the virally infected cells are human cells.
67. The method according to claim 56, wherein the virally infected cells are insect cells.
- 25 68. The method according to claim 56, wherein the uninfected host cells are eukaryotic cells.
- 30 69. The method according to claim 56, wherein the uninfected host cells are human cells.
70. The method according to claim 56, wherein the uninfected host cells are insect cells.

71. The method according to claim 56, wherein the virally infected cells are infected with recombinant virus.
- 5 72. The method according to claim 56, further comprising isolating viral products contained in and/or released from the incubated cells.
73. The method according to claim 72, further comprising lysing the incubated cells to release viral products prior to isolating viral products.
- 10 74. The method according to claim 72, wherein the viral products comprise a recombinant polypeptide.
75. The method according to claim 72, wherein the viral products comprise virus.
- 15 76. A method for selecting virally infected cells comprising:
inoculating a plurality of uninfected host cells with a plurality of viruses;
incubating the inoculated cells in the presence of incubation media
for a time and under conditions sufficient to obtain a plurality of
20 virally infected cells and monitoring the cell viability and average cell diameter of the incubated cells during the incubation; and
stopping the incubation at a point in time when the incubated cells have a viability of at least 50% and an average cell diameter that has increased by at least 0.5 μm .
- 25 77. The method for selecting virally infected cells according to claim 76, wherein the incubation is stopped at a point in time when the incubated cells have a viability of at least 90%.
- 30 78. The method for selecting virally infected cells according to claim 76, wherein the incubation is stopped at a point in time when the incubated cells have an average cell diameter that has increased by at least 1 μm .

79. The method for selecting virally infected cells according to claim 76, wherein the incubation is stopped at a point in time when the incubated cells have a viability of at least 90% and an average cell diameter that has increased by at least 1 μm .
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80. The method for selecting virally infected cells according to claim 76, wherein the monitoring comprises removing at least one aliquot of the incubating cells and determining the cell viability and average cell diameter of the cells in the aliquot in less than 10 minutes following removal of the aliquot.
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81. The method for selecting virally infected cells according to claim 80, wherein determining the cell viability and average cell diameter of the cells in the aliquot comprises placing at least a portion of the cells in an automated apparatus capable of measuring cell viability and average cell diameter of cells, and observing and/or recording the measured cell viability and the average cell diameter of the cells.
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82. The method for selecting virally infected cells according to claim 76, wherein the incubation is stopped by lowering the temperature of the incubated cells to 10 °C or lower.
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83. The method for selecting virally infected cells according to claim 76, wherein the incubation is stopped by centrifuging the incubated cells and incubation media to provide a pellet of cells and then removing the supernatant.
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